XLIV. THE FAT-SOLUBLE VITAMIN REQUIREMENTS OF THE CHICK.

I. THE VITAMIN A AND VITAMIN D CONTENT OF FISH MEAL AND MEAT MEAL.

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In a recent communication [McFarlane, Graham and Hall, 1930] it was shown that normal chicks could not be grown in confinement when fed synthetic diets which supplied what are believed to be adequate amounts of the vitamins known to be essential for growth in the chick. The birds during the 4th to the 6th week of growth developed certain deformities and paralysis of the limbs, a condition commonly designated as leg-weakness, the cause of which could not be adequately explained by what is now known of the nutritional requirements of the chick. Much difference of opinion exists in the literature in regard to the cause of this pathological condition, which has long been the greatest difficulty in rearing chicks in the laboratory on synthetic diets. It would appear either that more than one dietary factor may be responsible for this condition, or that there is more than one form of leg-weakness. One type of leg-weakness studied by many workers, notably Hughes and Titus [1926]; Mitchell, Kennard and Card [1923]; Hart, Halpin and Steenbock [1922]; Emmett and Peacock [1923] and more recently by Mussehl and Ackerson [1930] and by Massengale and Nussmeier [1930], can be prevented by the addition of cod-liver oil to the diet. As to whether this effect is due to vitamin A or vitamin D is still disputed. The preponderance of evidence indicates that it is a vitamin A deficiency which is the cause of this particular type of leg-weakness and that this leg deformity is not a rachitic condition [see Pappenheimer and Dunn, 1925]. All these investigators agree that the fat-soluble vitamin requirements of the chick are high and that to prevent the incidence of this form of leg-weakness, in chicks fed in the laboratory, at least 2 % of a high grade cod-liver oil must be added to the diet.

Plimmer, Rosedale and Raymond [1927] believe they have confirmed the findings of Dunn [1924] that as little as 0·5 % of cod-liver oil of good quality is sufficient to rear chicks to maturity. They obtained little or no growth and lost all the chicks within 4 weeks on feeding a diet composed of white rice

78 %, dried yeast 17 %, and fish meal 4 %. The addition of 0·4 or 0·6 parts of cod-liver oil (their Group XXXII A), did not materially improve the results. On changing the diet to white rice 75·3 %, dried yeast 16 %, fish meal 8 %, and cod-liver oil 0·5 % (their Group XLIII), excellent growth was obtained. They themselves express surprise that "birds could be reared on such small quantities of cod-liver oil as 0·1 and 0·2 %." Again with a diet composed of maize 89 %, fish meal 10 %, and cod-liver oil 1 %, normal rearing was obtained, but when the amount of cod-liver oil was reduced to 0·25 % or no cod-liver oil was given, rickets developed. The chicks, however, appear to have grown fairly well and only one chick was lost when the amount of cod-liver oil was as little as 0·25 %. From these results they have concluded that as little as 0·5 % of cod-liver oil of good quality is sufficient to rear chicks to maturity. Their conclusion is evidently based on the assumption that the fish meal contained no fat-soluble vitamins or possibly the fish meal had been freed from fat by extraction with ether, but this is not mentioned in their paper. The only essential difference between the diet fed to Group XXXII A which was a failure and that fed to Group XLIII which grew exceptionally well was in the fact that twice the quantity of fish meal was fed to the latter group; this improvement, we presume, they attributed entirely to the increase in the percentage protein in the diets. Again with the diet containing large quantities of maize, the failure of 10 % fish meal to protect the birds against rickets when no cod-liver oil was added may not indicate that fish meal contains little or no vitamin D but rather that maize compared with white rice, as shown by Mellanby [1929], contains a large amount of the anticalcifying factor which interferes with the deposition of calcium salts.

That fish meal may contain a very considerable amount of the fat-soluble vitamins while meat meal is not so endowed was suggested to us by the experiments of Graham and Smith [1929]. In a study of the influence of various protein materials on reproduction in the chick these workers found that the addition of 2 % of cod-liver oil to a diet for laying hens, containing 10 % of meat meal, had a pronounced effect in increasing the hatchability of the fertile eggs. The same basal diet but with 7·5 % of white fish meal as the protein supplement and without cod-liver oil gave just as high a percentage hatchability of the fertile eggs as that obtained with the meat meal diet containing 2 % cod-liver oil. The addition of 2 % cod-liver oil to the fish meal diet resulted in little or no improvement in the hatchability of the eggs.

The experiments to be described were undertaken to determine whether the vitamin A and vitamin D content of the fat of fish meal was different from that of the fat of meat meal. The results show conclusively that there is a difference, *i.e.* the particular white fish meal used in these experiments contained a very considerable amount of both vitamin A and vitamin D. In view of this the results of Plimmer *et al.* [1927] might legitimately be interpreted as showing that the vitamin A or vitamin D requirements of the chick are high and that the amount of vitamin A and vitamin D in the quantity of

fish meal fed in their experiments, while quite considerable, was not sufficient to protect the birds against rickets or a vitamin A deficiency, but that complete protection and normal growth were secured by adding 0·5 % cod-liver oil. Their findings, which constitute the most recent attempt recorded in the literature to assay the fat-soluble vitamin requirements of the growing chick, cannot, in the light of the results reported below, be considered reliable unless they have taken the precaution to remove by ether extraction the vitamin A and vitamin D which the fish meal used in their basal diet undoubtedly contains. Since this is not recorded in their paper, it can only be assumed that the fish meal added to their diet a considerable amount of the fat-soluble vitamins and that the vitamin A and vitamin D requirements of the chick may be relatively high instead of, as they have concluded, remarkably low.

EXPERIMENTAL.

The fish meal used in these experiments was a white fish meal prepared from non-oily fish such as cod (from which the liver had been removed) hake, plaice, haddock, skate and ling, etc. The meat meal was a finely ground meaty substance consisting of the residues of animal tissues but exclusive of hoof and horn and from which the fat had been partially extracted by hydraulic pressure after the direct drying of the material in revolving drums under 40 lbs. steam pressure (240°). The content of ether-soluble substances in fish meal is relatively low, while that in meat meal is much greater (see Table I). The cod-liver oil used was a high grade medicinal oil obtained from W. A. Munn, St John's, Newfoundland.

Table I. *Percentage composition of fish meal and meat meal.*

	Fish meal	Meat meal
Moisture	5·90	3·84
Crude protein (N × 6·25)	75·77	55·90
Ether extract	3·86	14·58
Crude fibre	0·23	1·22
Total ash	17·63	19·51
Silica	1·90	0·44
P_2O_5	5·55	6·85
CaO	5·97	8·55
MgO	0·88	0·59
Na_2O	1·81	1·02
K_2O	0·48	0·44
Fe_2O_3	0·016	0·04
Cu (mg. per kg.)	4·20	5·40
Ratio calcium : phosphorus	1·6 : 1	1·9 : 1

To ascertain whether fish meal or meat meal contains considerable amounts of vitamin A or vitamin D, the rates of growth of nine groups of barred rock chicks from 1 day to 8 weeks of age on the following diets were determined.

Fish meal groups.

Group I. Fish meal 14·8 %, marmite 15 %, white rice 70·2 %.

Group II. Same diet but with 2 % cod-liver oil replacing an equal amount of white rice.

Group III. Same diet as Group I, but with the chicks irradiated for 15 minutes daily from a quartz mercury vapour lamp at a height of 24 inches.

Group IV. Fish meal (freed from fat by extraction with ether) 14·6 %, marmite 15·0 %, white rice 67·4 %, and cod-liver oil 3 %.

Meat meal groups.

Group V. Meat meal 16·6 %, marmite 15 %, white rice 68·4 %.

Group VI. Same diet as group V but with 2 % cod-liver oil replacing an equal amount of white rice.

Group VII. Same diet as group V but with the chicks receiving ultra-violet irradiation for 15 minutes daily.

Group VIII. Meat meal (freed from fat by extraction with ether) 14·8 %, marmite 15·0 %, white rice 67·4 %, and cod-liver oil 3 %.

Control group.

Group IX. Caseinogen 12·2 %, marmite 15 %, white rice 65·8 %, cod-liver oil 3 %, and salt mixture 4 % [see Hart *et al.*, 1920].

The total protein (N × 6·25) of all these diets was approximately 18·5 %, being made up of 5·8 % from marmite, 2·6 % from white rice, and 10 % from each of the protein sources. The total ash of all the diets was approximately 4·4 %. The experiment was commenced with day-old chicks, 35 in each group, housed and managed in the laboratory under the same conditions as described by McFarlane, Graham and Hall [1930]. At the end of the 1st week the numbers were reduced to 30 chicks in each group, discarding those either showing evidence of constitutional weakness, or varying most widely from the mean weight of the group.

In Fig. 1 and Fig. 2 is shown the average growth in g. of the chicks receiving the different fish meal and meat meal diets. The weekly mortality in each group is also recorded on each growth curve in arabic figures, each value representing the number of chicks which died during the previous week. The growth of the control Group IX receiving caseinogen as the source of protein is recorded in each figure for comparison. No chicks were lost in this group during the first 8 weeks of growth.

We have also determined the calcium and inorganic phosphorus content of the blood-serum of a number of chicks in each group with the exception of Groups VII and VIII. In Fig. 3 and Fig. 4 are plotted the results of these determinations. The analyses at the end of the 1st week were made on the chicks which were being discarded from the experiment, it being found necessary to combine the blood from two chicks on the same diet to obtain sufficient blood for a determination. It was necessary to sacrifice two or three chicks in each group each week until the 8th, when sufficient blood for the analysis could be obtained from the brachial artery without the loss of the chick. Calcium was determined on 2 cc. of serum by the Clark-Collip modification [1925] of the Kramer-Tisdall method [1921]; inorganic phosphorus was determined on the supernatant fluid from the serum-calcium by the

Gunther-Greenberg modification [1929] of the Fiske and Subarrow procedure [1925]. For comparison we have also recorded the results of calcium and inorganic phosphorus determinations on the serum of chicks in the control caseinogen Group IX, of chicks receiving a rachitic diet and housed behind window-glass (Group X), and of chicks receiving the same diet as Group X but with 2 % of cod-liver oil added and housed under vita-glass (Group XI). The diet on which the chicks developed rickets was composed of yellow corn 37 %, ground wheat 50 %, oatmeal 25 %, buttermilk powder 10 %, meat

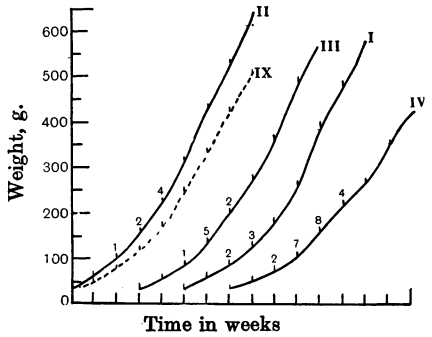

Fig. 1.

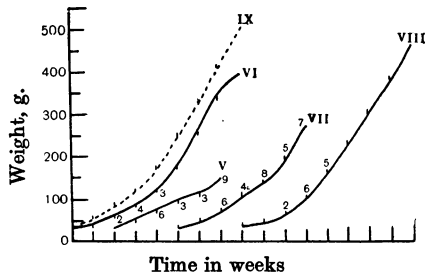

Fig. 2.

Fig. 1. The effect on growth of various additions to a diet composed of fish meal 14·8 %, marmite 15 %, and white rice to 100.

Curve I. No cod-liver oil added or ultra-violet irradiation.
„ II. 1 % cod-liver oil added.
„ III. Chicks received ultra-violet irradiation 15 min. daily.
„ IV. Ether-extracted fish meal and 3 % cod-liver oil.
„ IX. Caseinogen control diet and 3 % cod-liver oil.

Note. The arabic figures on each group curve in Figs. 1 and 2 give the number of chicks which died during the preceding week.

Fig. 2. The effect on growth of various additions to a diet composed of meat meal 16·6 %, marmite 15 %, and white rice to 100.

Curve V. No cod-liver oil added or ultra-violet irradiation.
„ VI. 1 % cod-liver oil added.
„ VII. Chicks received ultra-violet irradiation 15 min. daily.
„ VIII. Ether-extracted meat meal and 3 % cod-liver oil.
„ IX. Control caseinogen diet and 3 % cod-liver oil.

meal 12·5 %, alfalfa meal 3·5 %, bone meal 2·5 %, oyster shell 1·5 %, and salt 0·5 %.

Discussion.

During the first eight weeks of growth the inorganic phosphorus content of the blood-serum of normal chicks (Groups IX and XI, Fig. 4) is fairly constant but with chicks receiving a rachitic diet (Group X) the inorganic phosphorus content of the serum drops to a very low level during the 3rd and 4th weeks, when the birds show a typical rachitic condition, and then slowly recovers to the original level. These changes in the inorganic phosphorus of the blood-serum of rachitic chickens are similar to those accompanying uncomplicated mammalian rickets.

It will be seen from Fig. 1 that good growth and very little mortality was obtained with a diet containing fish meal as the only source of the fat-soluble vitamins (Group I) and that the result was practically unchanged when chicks on the same diet received ultra-violet irradiation (Group II). The growth was,

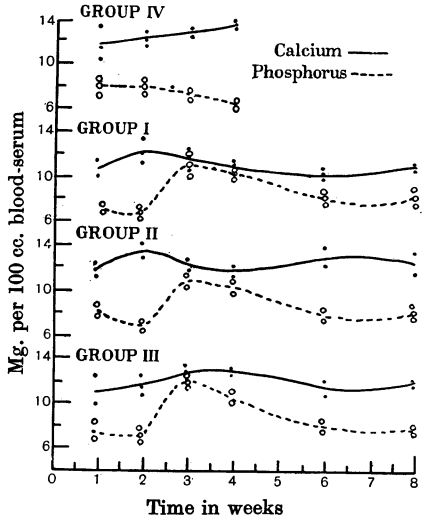

Fig. 3.

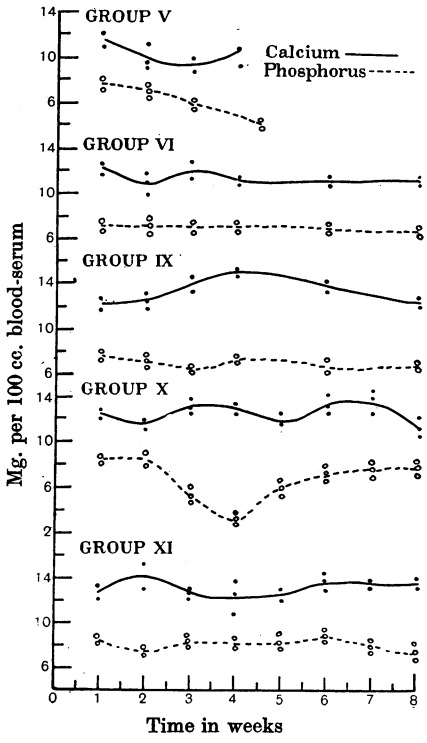

Fig. 4.

Fig. 3. Showing the effect on the calcium and inorganic phosphorus content of the blood-serum of chicks fed different fish meal diets.

Group I. No cod-liver oil. Group II. 1 % cod-liver oil.
„ III. Ultra-violet irradiation 15 min. daily.
„ IV. Fish meal ether-extracted and 3 % cod-liver oil.

Fig. 4. The effect on the calcium and inorganic phosphorus content of the blood-serum of chicks fed different meat meal diets.

Group V. No cod-liver oil. Group VI. 1 % cod-liver oil.
„ IX. Caseinogen control diet, 3 % cod-liver oil.
„ X. Rachitic diet, no cod-liver oil.
„ XI. Same diet as Group X but with 2 % cod-liver oil.

however, improved by adding 1 % cod-liver oil to the diet and was better than that attained by the caseinogen control Group IX. Fish meal which had been extracted with ether (Group IV) gave much the poorest growth of all notwithstanding the fact that 3 % cod-liver oil was added. It will also be observed that the mortality was very high with Group IV particularly during the 3rd and 4th weeks of growth. 50 % of these losses were the result of haemorrhage following the insertion of the identification bands into the wing.

Bleeding from the small wound slowly continued for 12 to 24 hours, the feather areas were continually wet with blood, the birds ultimately bleeding to death. The remainder of the losses all showed large sub-dermal haemorrhages along the femur (particularly the left), ribs and pectoral muscles and low blood volume due to haemorrhage. Blood from chicks in this group failed to clot on standing overnight in the laboratory. This condition was characteristic of the feeding of fish meal and to a less extent of meat meal which had been extracted with ether, no losses due to haemorrhage being recorded when caseinogen or untreated fish meal or meat meal was fed, although these chicks were all wing-banded at the same time and in exactly the same manner.

Coincident with the fact that the blood of the chicks fed ether-extracted fish meal would not clot there was also a distinct drop in the inorganic phosphorus content of the blood-serum (Fig. 3, Group IV) at the 4th week of growth. Unfortunately the losses in this group were so great that the blood-serum calcium and phosphorus determinations had to be discontinued at the end of the 4th week. However, the drop in the inorganic phosphorus is significant, particularly when it is noted (Fig. 3) that there was a very pronounced increase in the inorganic phosphorus content of the blood-serum during the 3rd and 4th weeks of growth of the chicks in all the groups fed unextracted fish meal. Since some factor other than the vitamin D, calcium or inorganic phosphorus content of the diet of the chick may have a profound effect on the concentration of inorganic phosphorus in the blood-serum during the first 8 weeks of growth, the value of this determination as a criterion of the presence or absence of rickets or leg-weakness in chicks is extremely questionable. It would seem that the removal of phospholipins from the fish meal was responsible for the result with Group IV but this does not explain the failure of the blood to coagulate, as the caseinogen of the control Group IX was also ether-extracted and the clotting time of the blood of the chicks was quite normal.

The results with these groups show that a diet containing approximately 15 % of this particular white fish meal as the only source of the fat-soluble vitamins contained sufficient vitamin A and vitamin D to give normal growth with no external evidence of either leg-weakness or rickets. The addition of 1 % cod-liver oil to this diet increased the rate of growth, presumably by increasing the vitamin A content of the diet, as increasing the concentration of vitamin D by ultra-violet irradiation had no significant effect.

With the meat meal groups (Fig. 2) the growth when no cod-liver oil was added (Group V) was extremely poor and all the chicks died within 5 weeks. The inorganic phosphorus content of the blood-serum of the chicks in this group fell rapidly during the first 4 weeks (Fig. 4, Group V). The chicks, however, did not grow sufficiently to show any external evidence of rickets. Giving the chicks ultra-violet irradiation resulted in an improved growth which, however, was still distinctly sub-normal and all the chicks were lost

within 6 weeks (see Fig. 2, Group VII). Adding 1 % cod-liver oil to the meat meal diet (Group VI), while greatly improving the results, still gave sub-normal growth when compared with the caseinogen control Group IX. Since normal growth was obtained with Group VIII receiving ether-extracted meat meal and 3 % cod-liver oil it does not seem probable that the nature of the protein or inorganic constituents was responsible for the sub-normal growth of Group VI. There is no significant difference in the composition of the ash of fish meal and meat meal so far as our analyses have been completed (see Table I). There were no external evidences of rickets in the chicks in Groups VI, VII and VIII. The inorganic phosphorus content of the serum of the chicks in the meat meal Group VI receiving 1 % cod-liver oil was constant throughout the first 8 weeks of growth.

The results with these groups show that a diet containing approximately 16·5 % of this particular meat meal as the sole source of the fat-soluble vitamins did not contain sufficient vitamin A or vitamin D to sustain growth. Since the addition of 1 % cod-liver oil to this diet still resulted in sub-normal growth and since normal growth was obtained when 3 % cod-liver oil was added to a diet containing ether-extracted fish meal it would appear that 1 % of high-grade medicinal cod-liver oil did not supply sufficient vitamin A or vitamin D to promote normal growth. Assuming the meat meal to be free from fat-soluble vitamins, the amount of vitamin A or vitamin D required to promote normal growth in chicks up to 8 weeks of age is, according to these results, in excess of the amount supplied by 1 % of high grade medicinal cod-liver oil.

Further work is being commenced to establish more accurately the vitamin A and vitamin D requirements of the chicks by varying the amount of cod-liver oil in the simplified caseinogen diet which has been used as a control in these experiments.

Summary.

1. A sample of white fish meal when fed at a level of 15 % in a diet composed of marmite and white rice was found to contain sufficient vitamin A and vitamin D to promote normal growth of chicks until 8 weeks of age. A sample of meat meal was found to contain little or no vitamin A or vitamin D when compared with fish meal.

2. The conclusion of Plimmer *et al.* [1927], that as little as 0·5 % of cod-liver oil of good quality is sufficient to rear chicks to maturity is based on the assumption that the fish meal used in their diets contained little or no fat-soluble vitamins. This assumption, for reasons discussed in the text, is believed to be erroneous. An experiment is described which shows that the amount of high grade medicinal cod-liver oil required to rear chicks, in the laboratory, to 8 weeks of age is in excess of 1 %.

3. Some factor, other than the vitamin D, calcium or inorganic phosphorus content of the diet of the chick, has been found profoundly to affect

the concentration of inorganic phosphorus in the blood-serum of chicks during the first 8 weeks of growth.

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